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ADAPTATION OF BRASSICA NAPUS SEEDLINGS TO VARIOUS BIOTIC AND ABIOTIC STRESSES UNDER DARK AND LIGHT REGIMEN

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Abstract

Roots of the plants are highly sensitive to interaction with abiotic or biotic components of the soil environment. During current study, 7 days old seedlings of hydroponically grown B. Napus were exposed to different biotic and abiotic stresses including AgNO3, Cinnamic acid, and Pseudomonas syringae under light and dark regimes. Also, the effect of different concentrations of nutrients was analysed by growing the B. Napus seedlings in 1 X, 0.5 X, 0.25 X of Hoagland solution. Data of root exudation of secondary metabolites with to respect to growth rate was noted for each treatment at the end of the week for each abiotic and biotic stress under dark and light at 24, 48 and 72 hours. It was noted that roots of dark grown seedlings exuded remarkably higher quantities of IAA and phenolics as compared to the control. Contrary to this, light exposure reduced the exudation of these secondary metabolites by B. Napus seedlings. Silver nitrate antagonistically affect the seedlings in dark due to the inhibition of the level of IAA, phenolics along with flavonoids and significantly reduced the growth rate. However, under the effect of 200mM cinnamic acid, the growth rate of seedling was improved with the inhibition of secondary metabolites in light condition. Interestingly, exudation of phytoalexins was not influenced conditions of light or dark while silver nitrate reduced its exudation from root in light. In the presence of CA, the light factor became an important modulator of phytoalexins exudation showing contrasting response. As expected, greater availability of nutrients(1 X Hoagland

solution) positively influenced the release of secondary metabolites by roots. The effect of 200Mm of AgNO3 and P. syringae was adverse on B. Napus as both inhibited the level of overall exudation of flavonoids and phytoalexins in dark grown seedlings.

Introduction

The secondary metabolites are the substances released by plants under stress conditions because these enable them to cope with environmental stresses (Munene, 2017). Some of these secondary metabolites include the family of auxin, flavonoids, phenolics, and alkaloids. Auxin is the primary growth hormone but it also has the secondary function in deciding the plant root interaction with the microbes. The concentration of free auxins fluctuates in the plant body exposed to environmental stresses (McClerklin, 2017). Due to this fluctuation, auxin exudes out of the plant root to establish

2 Adaptation of Brassica Napus Seedlins to varios biotic and abiotic stresses under dark and light Regimen the interaction with microbes in its vicinity. As large numbers of microbes are well-adapted to severe environmental stresses, due to the production of compatible solutes in them, plants intend to utilize their products to resist the stress. Therefore, under stress conditions, the plant is cliqued with the desirable microbe (Etesami, 2017).

Flavonoids are a group of chemicals that have remarkable antioxidant properties. Any oxidative burst due to the arrival of pathogen or abiotic stress is amended by these flavonoids as those reactive oxygen species which tend to oxidize the cell membrane and pave the way for entrance into the plant cells (George, 2017). On one hand, the flavonoids damage the pathogenic strategy of offense by scavenging the reactive oxygen species (ROS) and on another hand they also establish root interactions with beneficial microbes. Beneficial microbes communicate with the plants with proper chemical signalling, which impedes any restriction offered by the flavonoids (Gonzales, 2017). Unlike flavonoids, phenolics are utilized by plants to kill the microbes around them. Phenolics have the highest reducing property and are therefore used as a potent siderophore. Phenolics release the free iron which is readily absorbed by the roots.

The Majority of phytoalexins are alkaloids which are the group of waste chemicals produced as the end products of different metabolic activities (Hashimoto, 2017). The production of phytoalexins in the plant acts as the toxins for the attacking pathogen (Jeandet, 2017). They not only puncture the cell wall of the pathogen but also detain its maturation in a host. Phytoalexins deter the normal metabolism in both plant and the attacking; therefore they are only produced at the approach of a particular pathogen. The importance of phytoalexins is indicated by the increase of susceptibility of the host to pathogen. Those pathogens which can detoxify the phytoalexins are considered more virulent than those which are not capable to do so. They are involved in induced resistance which means they are produced under the effect of control hormonal expression (Yang, 2017). The most common hormonal expression in their production is salicylic acid, jasmonic acid, and ethylene.

The current status of plant science is mainly focused on plant microbial interactions. The plant releases secondary metabolites which decide the association of microbes with other plants. Therefore this study is intended to analyse the main secondary metabolite exudation from roots in light and dark under the effect of cinnamic acid and silver nitrate, and also to observe the effect of nutrient concentration on the exudation. Both the agents affect the exudation from roots but one promotes growth while the other inhibits it. The criteria of this study were also implemented on P. syringae stress on B. napus.

Methods and Materials

Seed sterilization and preparation of pots

Fresh seeds of B. napus were first primed with tap water for 2 h and then dipped sequentially in 0.1% solution of mercuric chloride for 10 seconds and 70% ethanol for 2 min. The seeds were then washed three times with autoclaved dH2O to wash off the traces of ethanol from the surface of the seeds. In order to initiate germination, the sterilized seeds were incubated in sealed petri plates at room temperature. After 2 days, when the seeds were sprouted, average sized seedlings were shifted to hydroponic media containing three replicates of each three concentrations of Hoagland solution (1X, 0.5X, 0.25X).

Bacterial cultures and inoculum preparation

For P. syringae culture, nutrient agar media and broth media were made in sterilized distilled water. The stock strain was taken in broth media overnight in shaking incubator at 25°C. The next day, bacteria were harvested by centrifugation at 3600 rpm for 20 min. Bacterial pellet was washed with autoclaved dH2O and then resuspended in 10 mM MgCl2 adjusted to an OD of 0.2 at 600 nm. The adjusted inoculum was cultured on agar media plates for counting colonies after overnight incubation at 25°C.

Growth measurements and metabolic analysis

2 days old seedlings of B. napus were grown in hydroponic media for 5 days in three different strengths (1X, 0.5X, and 0.25X) of Hoagland working solution under light and dark stress. Growth parameters of both conditioned seedlings including root length, shoot length and leaf area were measured to observe growth rate and samples of root exudes were also taken for metabolic profiling.

3 Adaptation of Brassica Napus Seedlins to varios biotic and abiotic stresses under dark and light Regimen In the 2nd phase, fresh 2 days old seedlings were again grown in hydroponic media following the same procedure but this time seedlings were treated with 200 mM of cinnamic acid and AgNO3 and with three different inoculums of P. syringae. Growth parameters were noted as above and samples of root exudes were taken for metabolic profiling. The metabolic profiling analyzed on spectrophotometer included:

1. Indole-3 Acetic acid (IAA) determination
2. Phenolics determination
3. Flavonoids determination

4. Phytoalexins determination

IAA was measured by taking 1.5 mL of centrifuged sample of root exudes, mixed with 2 mL of Salkowski's reagent and then kept in dark for 30 min. Optical density (OD) was measured at 540 nm on a photo-spectrometer. The ODs of the samples were analyzed with the IAA standard curve already made with standard solution of IAA on the same photo-spectrometer.

Phytoalexins in each root exudes were measured against the standard curve of camalexin when each sample of root exudes were treated with 2 mL of Mayer's reagent and 2 mL of 80% methanol. ODs were observed with photo-spectrometer using 450 nm and compared with camalexin standard curve. Total phenolics were observed by mixing 3 mL of centrifuged root exudes with 0.5 mL of Folin Ciokaltou reagent followed by incubation in the dark for 30 min. The optical density was measured by photo-spectrometer at 650 nm, and then the values were compared with standard curve of catechol. Similarly 0.5 mL of centrifuged root exudes was treated with 100 microliter of 10% AlCl3 and 10% potassium acetate. Finally, 4.3 mL of 70% ethanol was mixed with the samples and kept in dark. The ODs were measured using photo-spectrometer at 400 nm. The values were compared with the standard curve of quercetin.

Results and Discussion

Cinnamic acid signalled plant in light condition

Cinnamic acid is regarded as the potential inhibitor of secondary metabolites and is used as a protectant supplement against the most deleterious fungus like Fusarium oxysporum when produced endogenously in the plant (Gill, 2017). cinnamic acid is applied to 7 days old B. napus seedlings grown in three different concentrations of Hoagland solution (1X, 0.5X, 0.25X) in both dark and light conditions. The results indicated that the cinnamic acid has inhibited the total secondary metabolites level in the root exudes of seedlings grown in light condition after 72 h of the treatment (Figure 1). Previous studies have shown that cinnamic acid interferes with most hormonal efflux into the target cell, of which the most noticeable is IAA (Deinum, 2016). Our study shows that cinnamic acid triggered the B. napus 7 days old seedlings in light condition by inhibiting the IAA and flavonoids. A cinnamic acid is an inhibitor of IAA efflux, thus considering the plant health, it also promotes the root growth (Steenackers, 2016). Our study, in this case, revealed that the effect of same concentration of cinnamic acid has inhibited the growth of root in dark while in light it promoted the growth of the same seedlings (Figure 1e). This scaffolds our assumption more that cinnamic acid triggers plant growth in light conditions. However, there is no decrease in secondary metabolites in root exudates after 24 h. This reflects that signalling of the cinnamic acid is quite sluggish or there is a first line defence of plant which withstood the effect of cinnamic acid. The way to operate the level of exogenous cinnamic acid against the fungus which paves its infection using the modulation in IAA level in the plant can also be the goal of future research. In plants, hormonal signalling occurs when there is enough light as the light involves in ETC, generates enough chemical energy to run its physiology. But the puzzling role of cinnamic acid is the inhibition of flavonoids in plants. More interestingly, its effect also varied with the varied Hoagland concentrations (Figures 1). Flavonoid has the primary function to dissolve the phosphate ion in the soil, therefore higher the phosphate is incorporated in the medium, higher will be the exudation of flavonoids (Figure 1d). Cinnamic acid inhibited its exudation more on 1X which means the B. napus has overcome its effect as the concentration of phosphate ion increases in the solution, maintaining the normal growth of the seedlings. This also refers that the immature seedlings of 7 days is under stress of 1X as the

 concentration of nutrients are high as required by normal growth of 7 days old seedlings of B. napus. Moreover, if cinnamic acid is considered to be the only defensive substance against any pathogenic microbe, the perception can be wrong (Mithöfer, 2017). Flavonoids is the first line defense of plant to any microbial attack. Therefore, something is missing between the antagonistic relation with flavonoids and cinnamic acid and the work should be done on it in future. As in 0.25X, the effect of cinnamic acid was found to be highly inducible which may be referred as the lower concentration of iron in the solution, concluding its similarity with siderophore (Figure 1c).

Total phenolic contents remained unaffected as compared with IAA and flavonoids. However, the level of phenolics was lower in light as compared to dark contrary to the cinnamic acid, which inhibited flavonoids and IAA in light. Phenolics cannot be taken in tothe account for this inhibition as phenols get oxidized readily in light (Chowdhury, 2017). Regarding the role of phytoalexins, cinnamic acid affects its level in dark than light at 24hr and the subsequent. It is the one among four groups of secondary metabolites which remained unaffected in light and reduced in dark. However, under normal dark and light conditions with any of the concentration of Hoagland working solution, the phytoalexins remained unaffected. It reflected that B. napus produce it without any effect of the differences of nutrients in growing media but cinnamic acid reduced its level in dark. Previous studies provide no clue against it.

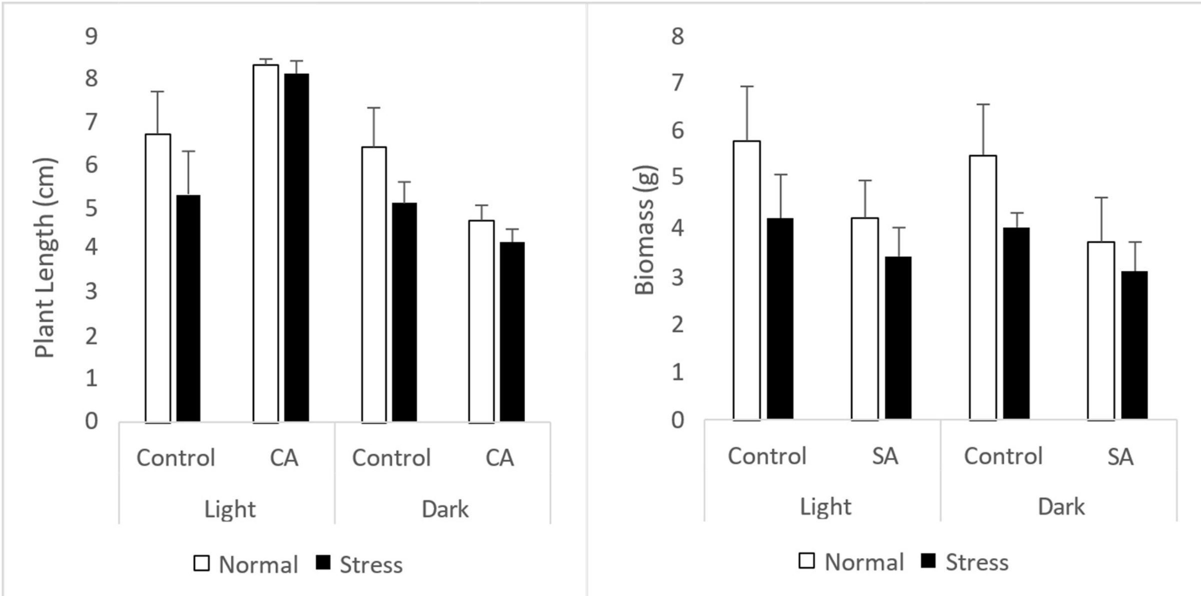
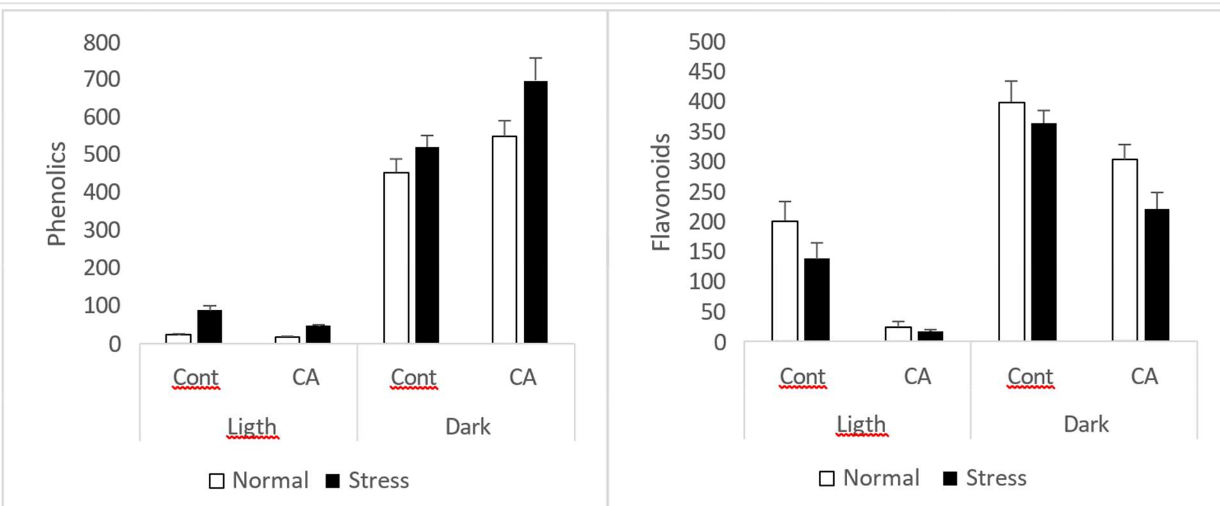


Figure 1 (a-b)

Effect of cinnamic acid on the plant growth and exudation of secondary metabolites from root of 7 days old B. napus seedlings was studied. The cinnamic acid has improved growth and biomass of the seedling under light condition (a, b),



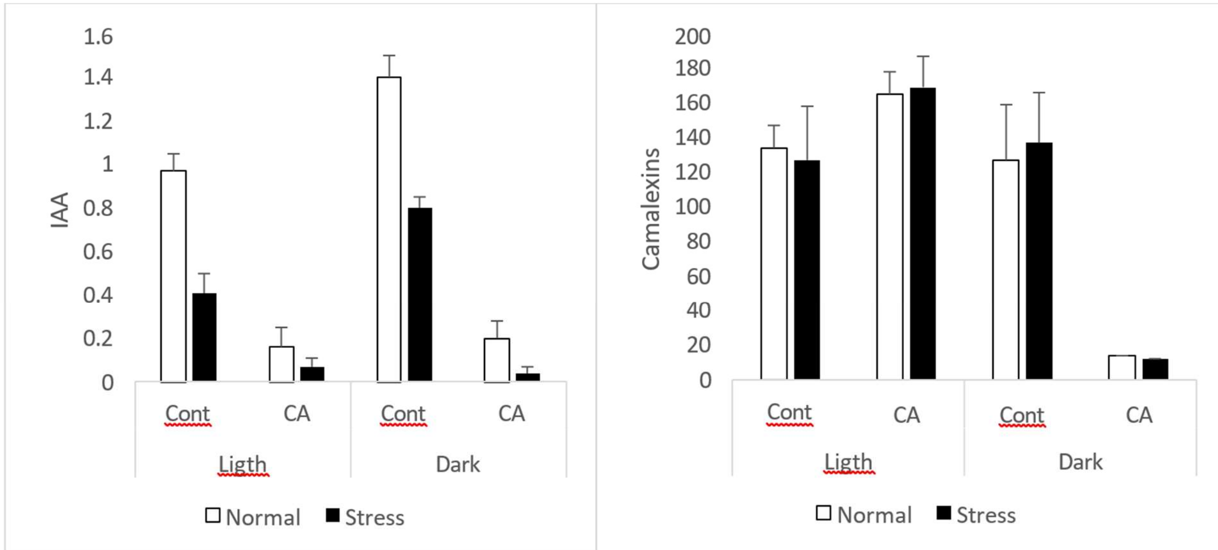


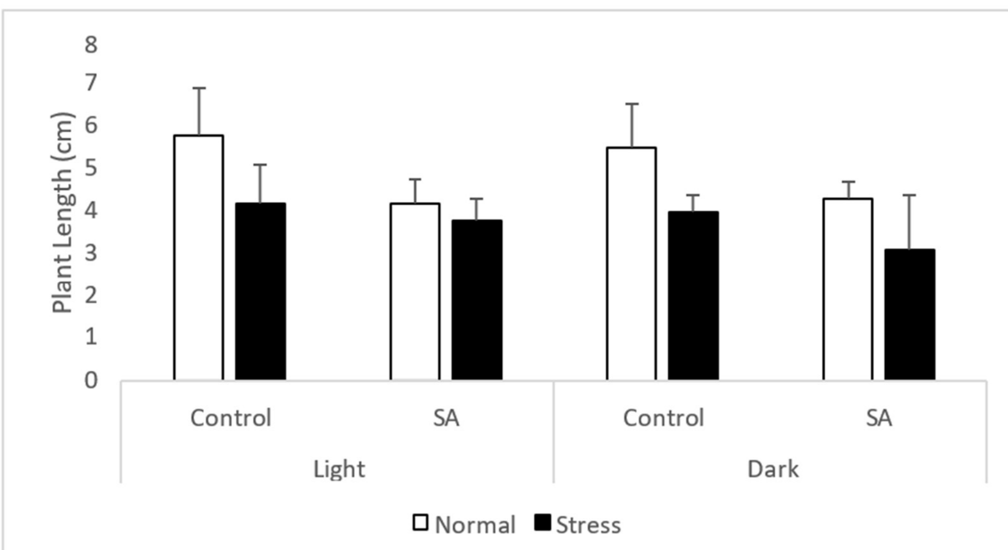
Figure 1 (c-f)

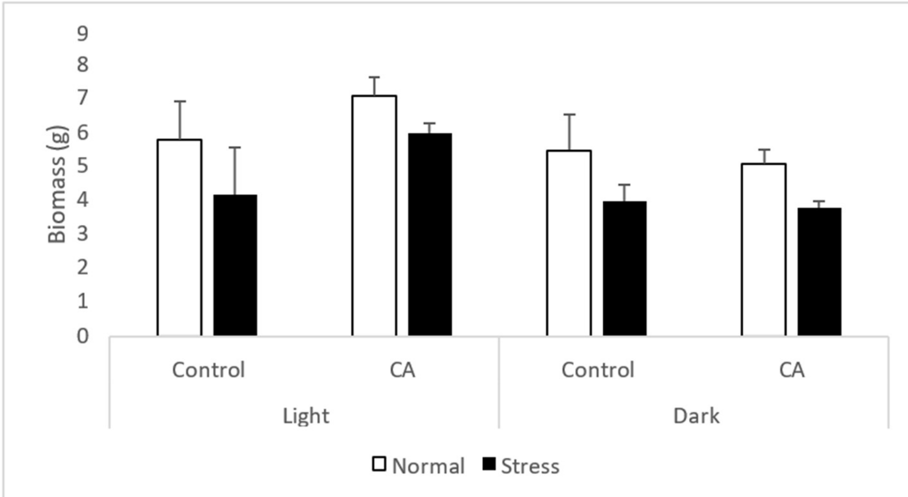
The cinnamic acid inhibited the total IAA, flavonoids contents were inhibited under light and dark condition in normal and low nutrient concentration (d, e) where phenolic contents were inhibited in normal nutrient concentration; in stress, there was no effect of cinnamic acid in it (c). Cinnamic acid affected the level of camalexin in dark while in light its exudation from root was promoted (f).

Silver nitrate triggered plant in dark condition

Silver nitrate is known for having a phytotoxic effect on plants and silver nanoparticles are used by many phytopathogens as an agent for virulence (Vishwakarma, 2017). 200mM solution of silver nitrate was applied on 7 days old seedlings of B. napus under light and dark and then their root exudes were analyzed and considered relatively with the growth of the seedlings (Figure 2). Silver nitrate under dark , inhibited the total root exudes in the 24, 48 and 72 h of treatment which had flavonoid content, phenolics, and IAA while in light there was no inhibition but the phytoalexins. This strongly suggestedthat 7 days old B. napus seedlings had overcome the phytotoxic effects under light. Silver nitrate produces oxidative stress by the induction of super oxide radicles and hydrogen peroxide which undergo DNA degradation and ultimately cell death (Mythili, 2017). Interestingly, our finding on B. napus seedlings had showed that under light condition, the concentration of root exudes was normal as compared to dark treated in same seedlings. Therefore, it is more convenient to say that accompanied with dark stress, the growth seedlings of B. napus of 7 days old was compensated even with low exudation of root. Plants exude secondary metabolites to nullify the oxidative damage or in response to communication with an agent in the soil to cope with the nutrient deficiency in growth media (Cortes-Morales, 2017). In this regard, our results revealed that the seedlings did not produce the desiredamount of antioxidant flavonoids and phenolics which could diminish the deleterious effect of silver nitrate and most of the seedlings showed excessive loss in the total rate of growth. It is the known fact about the plant root exudation of flavonoids and phenolics under dark to manipulate the microbial growth around its root zone as most of the microbes are hyperactive in dark condition (Van der Wal, 2017), but silver nitrate strongly inhibited the seedlings of B. Napus from normal growth and exudation of such antioxidant compound. The resultant seedlings were highly reduced in growth. IAA as the secondary metabolite acts as the communication medium that attracts the beneficial microbes in growth media ((Aguirre-Becerra et al., 2021)). In our results, silver nitrate also inhibited the IAA level as the secondary metabolite which deteriorated the conditions for B. napus 7 day's old seedlings under dark. Under light, the level of IAA as a secondary metabolite was high. Therefore, the growth of the seedlings was improved. The suggestion in our finding to ameliorate the effect of silver nitrate is the high concentration of flavonoids and phenolics in growth media. Considering the concentrations of Hoagland solution, the inhibition of total root exudes occurs in 0.25X, while 0.5X plays the transition role between 1X and 0.25X. The inhibition of flavonoid is understandable due to the low concentration of phosphate ions in the solutions but the phenolics which are incorporated with

6 Adaptation of Brassica Napus Seedlins to varios biotic and abiotic stresses under dark and light Regimen low concentration of iron in the media were also low. Phenolics act as the siderophore and are released by the plants when there is deficiency of iron of in growth media (Cesco, Neumann, Tomasi, Pinton, & Weisskopf, 2010)). This refers that silver nitrate only strongly inhibits the phenolics among the four secondary metbolites. Phytoalexins were remained unchanged in dark condition in 7 days old seedlings of B. napus but the previous studies concluded that silver nitrate is the strong inhibitor of phytoalexins. There was a slight decrease in the exudation of phytoalexins under light condition in 0.5X as compared to 1X and 0.25X which could be the question to address in our future research.

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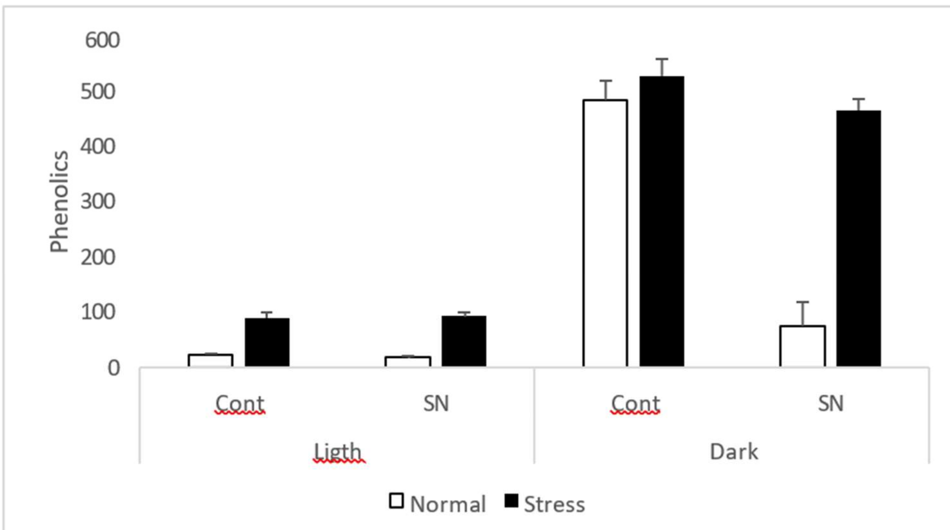
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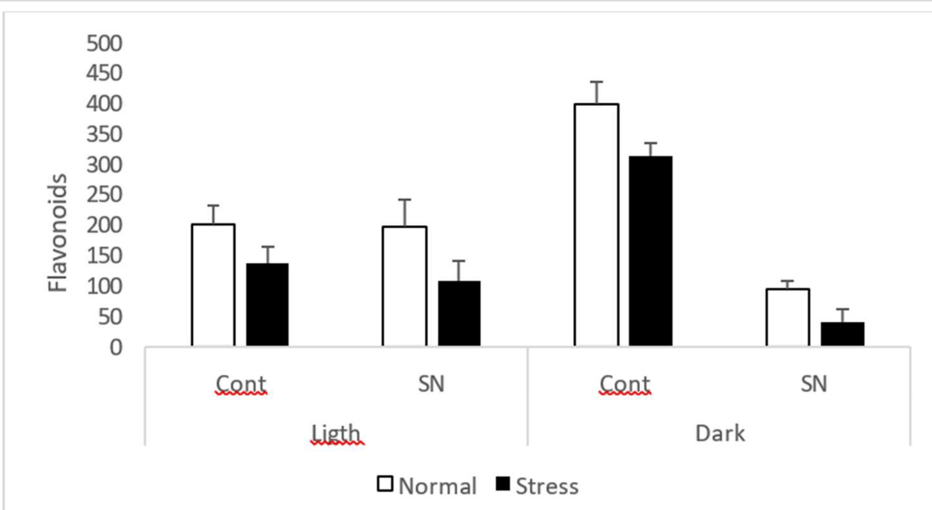
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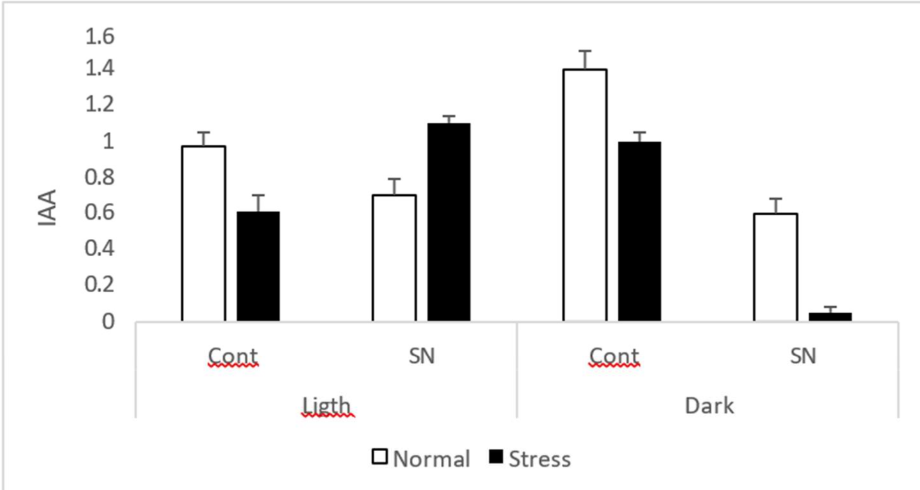


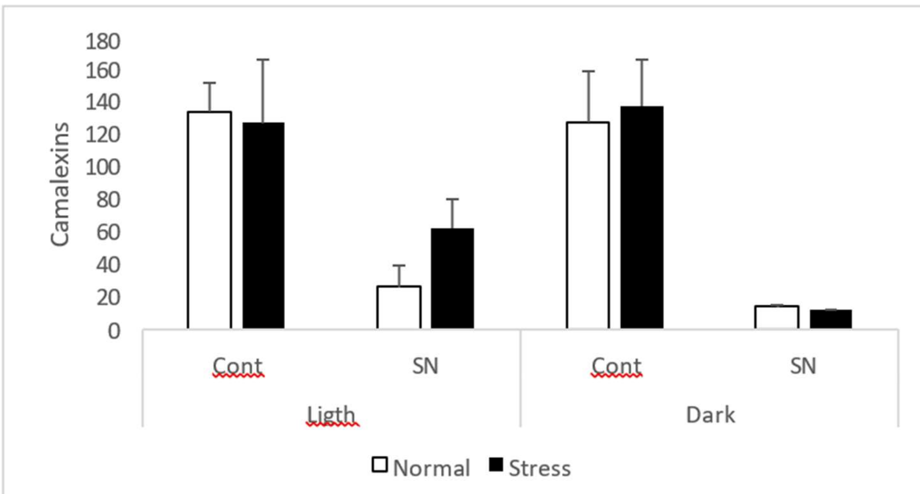


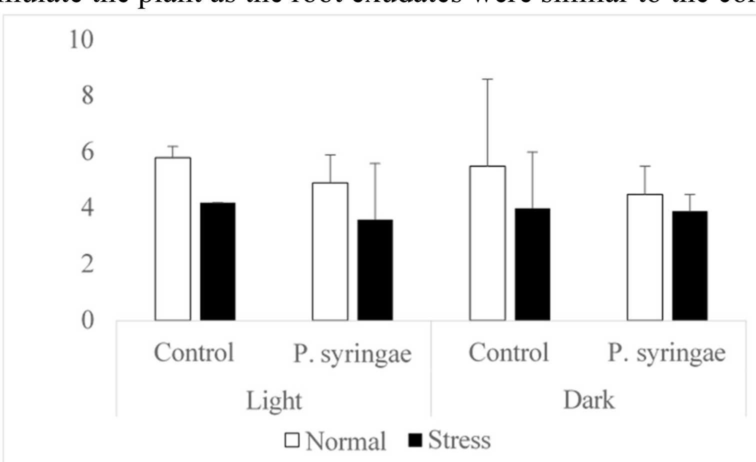
Figure 2

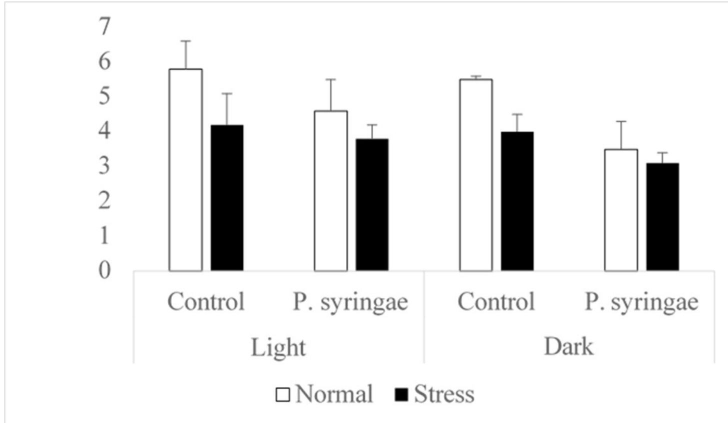
Effect of silver nitrate on the plant growth and exudation of secondary metabolites from root of 7 days old B. Napus seedlings was studied. The silver nitrate has degraded growth and biomass of the seedling under dark condition (a, b), and it inhibited the total IAA, flavonoids contents were inhibited under dark condition in normal and low nutrient concentration (d, e) whereas phenolic contents were remained unchanged in normal nutrient concentration and in stress(c). Silver nitrate triggered the level of camalexin in light while in dark it appeared its inhibitor (f).

CFU 106 is optimal to study the effect of P. syringae on plant

The Rhizosphere of the plant is surrounded by many biological processes which are collectively referred to as hormonal communication with the microbes (Nath, 2017). This communication either leads to the detachment of the microbe which acts as a pathogen or attractant of the beneficial microbe in a stressed condition. P. syringae is the well-known bacterial paradigm that triggers the plant to act against it. P. syringae in three different CFU concentrations is being applied on 7 days old seedlings of B. napus grown in the three concentration of Hoagland solution (1X, 0.5X, and 0.25X) under dark and light condition to analyse its effect on the seedlings. Our result deduced

that CFU in a concentration of 106 acts as optimal to study its effect on the seedling. The total concentration of root exudes was inhibited after 48 h of the incubation except IAA. IAA was only the secondary metabolite which showed the progressive increase in incubation duration (24h, 48h and 72h) under dark. Phytoalexins were high in 24 h but with the later phases of infection, the metabolites were inhibited. This reflected that phytoalexins have the role in active plant defense. The role of phenolics in this case is quite ambiguous as there was high concentration of phenolics in root exudate under dark but taking the plant
health it was poor due to the severe infection. This concluded that P. syringae released a substance as a nanoparticle which diminished the effect of phenolics and solely on IAA, P. syringae affected the seedlings. Under light, the seedlings were grown well and there was normal concentration of secondary metabolites in root exudates. Some microbes are called the dark world which means they only affect the plant in dark condition (Poole, 2017). P. syringae on 7 days old seedlings are called as dark world as discussed by its effects in dark. Moreover in CFU 103, the seedlings remained dead after 24 h of incubation when the root exudates of the seedlings were analyzed, there was high concentration of phenolics and IAA while the flavonoids and phytoalexins were inhibited by the bacterial strain under dark condition. If the same case was taken for the seedlings grown under light condition, there was a low concentration of IAA and phenolics and the seedlings were in poor health condition. A higher concentration of calcium in the growth media of plant uses as the deterrence against pathogenesis as calcium undergo Calreticulins signalling (Sarwat, 2017) Regarding to the concentrations of Hoagland solution, it was observed that for 1X, the infection was not that severe as compared to 0.5 X but in 0.25 X, the infection was too severe. The high infection in 1X is still an enigma to be solved in the further research. It may be suggested that phenolics does not have role in antibacterial activity or P. syringae has released strong oxidizing agents as the virulent factor which diminished the antibacterial effect of phenolics. CFU 109 of P. syringae did not simulate the plant as the root exudates were similar to the control.

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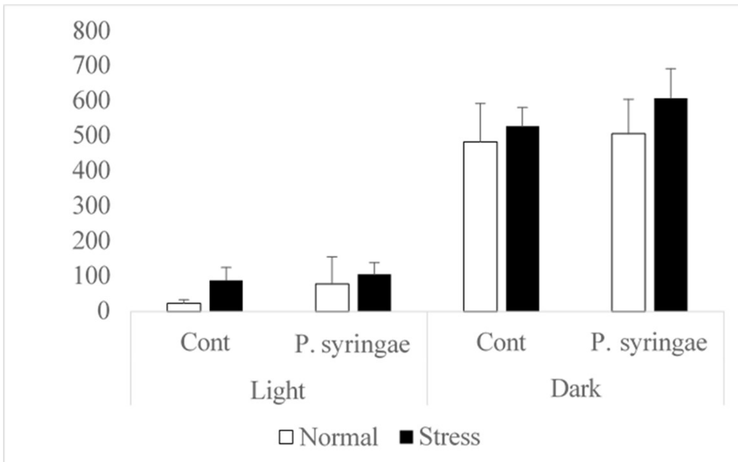
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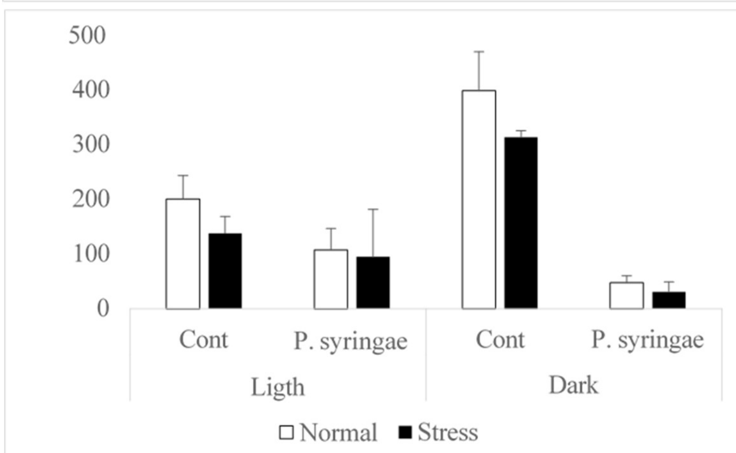
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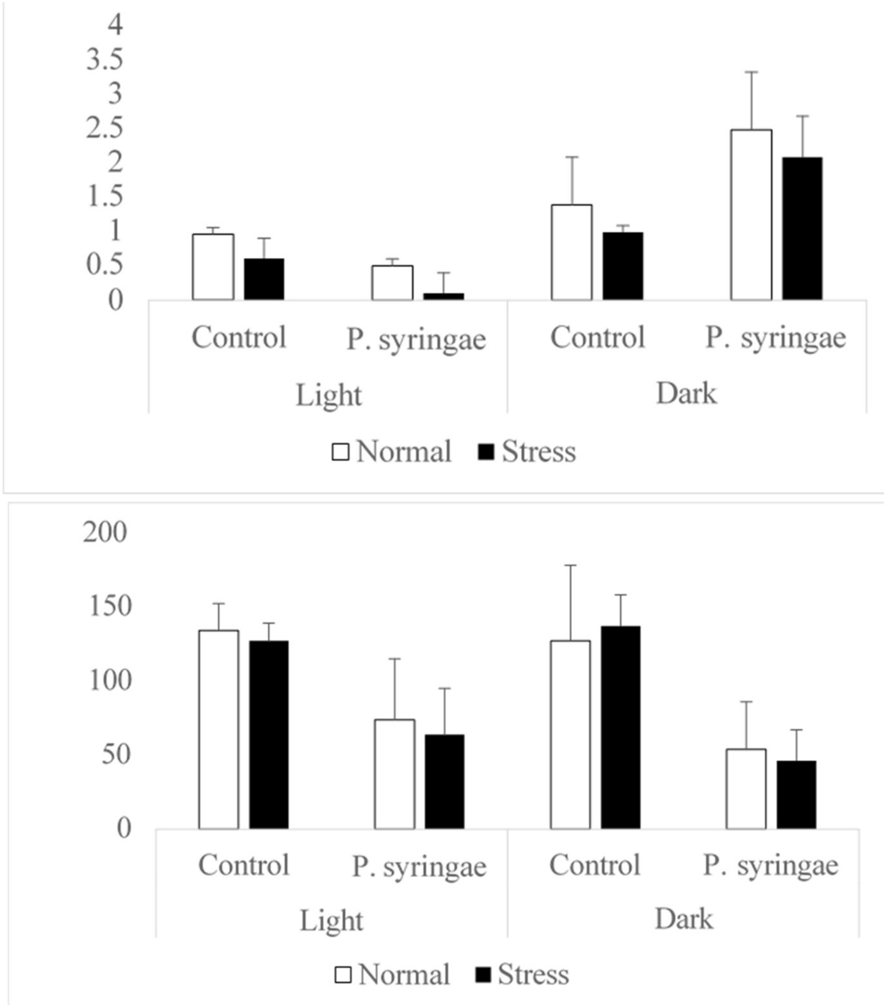

Figure 3 Effect of P. syringae on the plant growth and exudation of secondary metabolites from root of 7 days old B. Napus seedlings was studied. P. syringae has degraded growth and biomass of the seedling under dark condition and light condition (a, b), and it inhibited the total IAA, and camalexin contents were inhibited under dark condition in normal and low nutrient concentration (d, e) whereas phenolic contents were remained unchanged in normal nutrient concentration and in stress(c).

Conclusion

B. napus 7 day old seedlings were highly inducible to any environmental stress as the effect of cinnamic acid, silver nitrate, and P. syringae were studied. Cinnamic acid is the organic compound also produced in plants endogenously. It triggered the seedlings in light conditions as the health of seedlings was quietly improved as it also inhibited the exudation from the roots of most secondary metabolites. Silver nitrate is another inhibitor of secondary metabolites but it adversely affected the health of seedlings whereas inhibiting the exudation of secondary metabolites and acted as a strong oxidizing agent. The effect of silver nitrate on the 7 days old seedlings was more noticeable in dark. However in light, the exudation of secondary metabolites in root exudates had nullified the effect of silver nitrate. P. syringae effect on the seedlings was also adverse as it inhibited the total flavonoid content and phytoalexins. However the level of IAA and phenolics was high. The infection of P. syringae was solely dependent on the inhibition of flavonoids and phytoalexins and the induction of phenolics in B. napus seedlings. Therefore it is concluded that the seedlings were highly inducible to any sstress response and regarded as optimal source to study the biotic and abiotic stress.

Acknowledgements
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